SSB-1 of the Yeast *Saccharomyces cerevisiae* Is a Nucleolar-specific, Silver-binding Protein That Is Associated with the snR10 and snR11 Small Nuclear RNAs

Michael W. Clark,* Man Lun R. Yip,* Judith Campbell,‡ and John Abelson*

Divisions of *Biology and ‡Chemistry and Biology, California Institute of Technology, Pasadena, California 91125

Abstract. SSB-1, the yeast single-strand RNA-binding protein, is demonstrated to be a yeast nucleolar-specific, silver-binding protein. In double-label immunofluorescence microscopy experiments antibodies to two other nucleolar proteins, RNA Pol I 190-kD and fibrillarin, were used to reveal the site of rRNA transcription; i.e., the fibrillar region of the nucleolus. SSB-1 colocalized with fibrillarin in a double-label immunofluorescence mapping experiment to the yeast nucleolus. SSB-1 is located, though, over a wider region of the nucleolus than the transcription site marker. Immunoprecipitations of yeast cell extracts with the SSB-1 antibody reveal that in 150 mM NaCl SSB-1 is bound to two small nuclear RNAs (snRNAs). These yeast snRNAs are snR10 and snR11, with snR10 being predominant. Since snR10 has been implicated in pre-rRNA processing, the association of SSB-1 and snR10 into a nucleolar snRNP particle indicates SSB-1 involvement in rRNA processing as well. Also, another yeast protein, SSB-36-kD, isolated by single-strand DNA chromatography, is shown to bind silver under the conditions used for nucleolar-specific staining. It is, most likely, another yeast nucleolar protein.

EUKARYOTIC ribosomal RNA transcription and processing occurs in concert with the assembly of the ribosome. These complex processes are carried out in the nuclear subcompartment, the nucleolus (39). The nucleolus is a multicomponent complex with an intricate, yet highly ordered internal structure that appears to have direct involvement in nucleolar functions. To fully understand the coordinate control of these complicated, but temporally ordered, cellular activities, the composition and spatial arrangement of the nucleolar components must be demonstrated. Ultrastructural studies of the vertebrate nucleolus show this structure to be divided into three distinct regions that contain separate functions (11): the fibrillar center, which is the site of rRNA transcription (34); a dense fibrillar region bounding the fibrillar center, which is the area of precursor rRNA accumulation and the initial stages of precursor rRNA processing (9) (although there is some controversy on this point [4]); and, finally, a granular region, which is the site of pre-rRNA processing and precursor ribosome assembly (15, 16).

Biochemical investigations of the vertebrate nucleolus have revealed a number of resident nucleolar components, which were not ribosomal proteins or rRNAs, that appeared to be involved in the control and maintenance of nucleolar structure and function (for review see reference 39). Three of the most well-studied mammalian nucleolar proteins are fibrillarin, nucleolin, and B23, (23, 24, 26, 40). Although these proteins are located in different nucleolar regions and thus appear to be involved in different nucleolar activities, they still share characteristics common to many single-strand RNA (ssRNA)[1]-binding proteins, particularly the RNP consensus sequences (2, 6, 30) and a glycine-arginine-rich repeated motif (24). A unique feature of some of these nucleolar proteins is the ability to specifically bind silver under acidic conditions in which most other cellular proteins remain unstained (23). Such nucleolar-specific, silver-binding proteins have been found in all eukaryotic organisms (23, 24, 26, 40).

Another characteristic shared among eukaryotic nucleoli is that, like precursor mRNA processing events, rRNA maturation and ribosome assembly appears to be directed by protein–small nuclear RNA (snRNA) complexes referred to as snRNP particles. Immunoprecipitation of mammalian nuclear extracts with antibodies to the nucleolar protein fibrillarin will precipitate three snRNAs: U3, U8, and U13. It is thought that fibrillarin forms separate snRNPs with the three snRNAs (45). In vitro hybridization and nucleotide sequence comparisons (27) with the U3 snRNA and the pre-rRNA revealed that U3 snRNA can interact with at least three regions of the pre-rRNA. These data implicated the fibrillarin

M. W. Clark's present address is Department of Biology, McGill University, Montreal, Quebec, Canada, H3A 1B1.

1. *Abbreviations used in this paper*: R.T., room temperature; snRNA, small nuclear RNA; ssRNA, single-strand RNA.

snRNP in the nucleolar functions of pre-rRNA processing or pre-ribosome assembly. snRNA involvement in these nucleolar functions appears to be an evolutionarily conserved feature extending to the yeast *Saccharomyces cerevisiae*. By similar immunoprecipitation studies and gene disruption experiments in yeast, a set of the yeast snRNAs has also been implicated in ribosome biogenesis. These yeast snRNAs are snR3, 4, 5, 8, 9, 10, 17, 128, 190 (28, 42, 43).

The highly developed techniques of molecular biology and genetics available in yeast make this organism very amenable to the investigation of how the distinct morphology of the nucleolus impacts on proper nucleolar functioning. Furthermore, the relative simplicity and nuclear position of the single yeast nucleolus, which appears as a caplike structure juxtaposed to the nuclear envelope (5), readily lends it to morphological studies. The nucleolus of yeast shows less well-defined nucleolar segregation at an ultrastructural level than the nucleolus of more complex organisms. The yeast nucleolus can be seen to have only a fibrillar and a granular component containing no region similar to the dense fibrillar region seen in more complex cells (13; Clark, M. W., personal observation). A small number of yeast nucleolar proteins have been identified and reported. These proteins are RNA Pol I 190-kD (36), SSB-1 (20), fibrillarin (7, 14, 35), p90 (14), p40 (14, 17), and p38 (1, 47). In this paper we further characterize SSB-1, which is a nucleolar resident, nonribosomal protein.

Previously, SSB-1 was found to possess many of the characteristics common to a nucleolar protein. It is an ssDNA/RNA-binding protein with a molecular weight of 32,800 and a pI of 6 (20). It contains amino acid sequences similar to the RNP consensus sequences found in almost all ssRNA-binding proteins (2, 6, 30). SSB-1 also contains a small repeating stretch of glycine, arginine, and phenylalanine that resembles a repeating motif found in nucleolin and fibrillarin (20, 24, 35). SSB-1 was shown by immunofluorescence staining to have the same staining patterns as the yeast nucleolus. It was thus thought to likely be a nucleolar localized protein (20). We show here that the SSB-1 protein is one of the yeast nucleolar-specific, silver-binding proteins. A more definitive nucleolar location of SSB-1 is demonstrated using double-label immunofluorescence microscopy with the antibody to SSB-1 and the human autoimmune serum to fibrillarin. The fibrillarin autoimmune serums reacts with a 36,000-mol wt yeast protein. This protein is shown to reside in the fibrillar region of the nucleolus; i.e., the site of yeast rRNA transcription. This antifibrillarin serum is used as a marker for the fibrillar region of the yeast nucleolus. In this manner SSB-1 is mapped to the nucleolus. SSB-1 appears to have involvement in rRNA processing or pre-ribosome assembly, demonstrated by immunoprecipitation with the SSB-1 antibody. In immunoprecipitation experiments SSB-1 binds strongly to two yeast snRNAs, snR10 and snR11. snR10 has been shown by Tollervey (42) to have direct action in the pre-rRNA processing pathway. Interestingly, the snRNA binding specificity of SSB-1 is salt dependent; in high NaCl concentrations SSB-1 binds to other snRNAs. The salt-dependent binding preference is a characteristic that this yeast nucleolar snRNP shares with the mammalian fibrillarin U3 snRNP (45).

Materials and Methods

Materials

Anti-rabbit IgG-FITC and -TRITC conjugates, and the human antifibrillarin serum as ANA-nucleolar staining antibody were purchased from Sigma Chemical Co., St. Louis, MO. Anti-human IgG-FITC conjugate and low molecular weight SDS gel protein markers were purchased from Bethesda Research Laboratories, Bethesda, MD. Vector ABC anti-rabbit IgG and anti-human IgG kits were purchased from Vector Laboratories, Inc., Burlingame, CA. Restriction endonucleases were purchased from New England Biolabs (Beverly, MA). All chemical reagents were purchased from J. T. Baker Chemical Co., Phillipsburg, NJ. T4 RNA ligase and protein G-Sepharose 4 FF were purchased from Pharmacia Fine Chemicals, Piscataway, NJ. The random-primed DNA labeling kit was purchased from Boehringer Mannheim Diagnostics, Inc., Houston, TX. Nitrocellulose was purchased from Schleicher & Schuell, Inc., Keene, NH. 32pCp and GeneScreen were purchased from New England Nuclear, Boston, MA. RNAsin was purchased from Promega Biotic, Madison, WI.

Strains and Plasmids

Yeast strain EJ101, a protease-deficient strain (α-pep4-3, prc1, prb1, his) used for the immunofluorescence microscopy, was obtained from E. Jones. Yeast strain K4/YM214 (α his3delta200 lys2-801 adel-101 ura3-52) which contained the plasmid pSB1-4 (SSB-1 gene 6.4-kb fragment in Yep24), which was also used in immunofluorescence microscopy, was obtained from A. Jong.

Immunofluorescence Microscopy

Immunofluorescence techniques were done as described in Clark and Abelson (10) for pre- and postfixed whole mount yeast cells.

Postfixed Cells

200 ml of yeast culture was grown to an OD_{600} <0.5. Cells were harvested by filtration through a 0.45-μm filtration unit (Nalge Co., Rochester, NY). Cells were washed once with 10 ml of ice-cold distilled water and twice with 10 ml of 0.1 M K-phosphate, pH 6.5. The cells were resuspended in 1 ml of 100 mM Tris-HCl, pH 8, 25 mM DTT, 5 mM Na_2-EDTA, 1 M sorbitol, and incubated at 30°C for 10 min. Cells were pelleted by centrifugation and that pellet was washed in 5 ml of 0.1 M K/phosphate/citrate, pH 5.8, 1.2 M sorbitol. The cells pellet was resuspended in 0.5 ml of the same buffer. 0.05 ml of β-glucuronidase (type H2) and 0.05 ml of a 5-mg/ml solution of zymolyase were added. This cell suspension was incubated at 30°C for 1.5-2 h to thoroughly remove cell walls. The cells were centrifuged and the pellet was washed twice with 0.1 M K/phosphate/citrate, pH 6.5, 1.2 M sorbitol. The pellet was resuspended in the same buffer at an OD_{600} of 0.5. 0.1 ml of the cell suspension was applied to a polylysine-coated microscope slide and permitted to stand for 5 min while the cells settled. The remaining supernatant was then removed. The cells were then fixed by the application of 5% formaldehyde in 0.1 M K/phosphate/citrate, pH 6.5, 1.2 M sorbitol for 10 min. The supernatant was removed, the cells were washed once with the buffer alone, and then the slides were immersed in −20°C methanol for 6 min followed by two 30-s immersions in −20°C acetone. The slides were then air dried and stored at 6°C until needed.

Pre-fixed Cells

This procedure is the same as above except that after the cells were harvested by filtration and washed they were fixed by resuspension in 3.7% formaldehyde in 0.1 M K/phosphate, pH 6.5, and incubated for 1.5 h at room temperature (R. T.). After fixation, the cells were washed four times in the buffer alone to remove the formaldehyde. The cells were then treated as postfixed cells, except the fixation procedure after mounting the cells on the slides was omitted.

The Immunofluorescence Staining

This technique was done as described in the figure legends. The area of the fluorescence staining was determined by measuring photomicrographs on

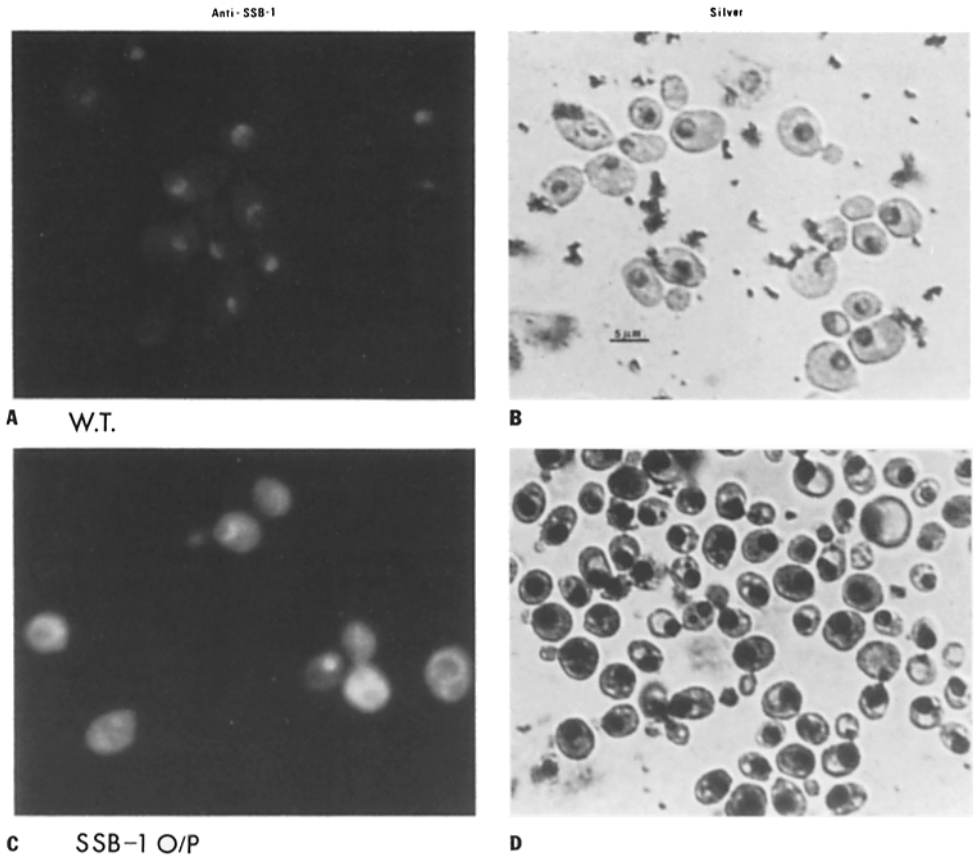

Figure 1. Staining of yeast wild-type cells (*A* and *B*) and a strain of yeast which overexpresses SSB-1 threefold over wild-type levels (*C* and *D*). *A* and *C* were stained for immunofluorescence with an affinity-purified antibody to SSB-1. *B* and *D* were stained with the nucleolar-specific silver stain procedure (20). With overexpression of SSB-1, both SSB-1 antibody fluorescence and nucleolar-specific silver staining were seen not just in the nucleolus but over the entire cell. Bar, 5 μm.

a digitizing tablet connected to a personal computer containing the Jandel "Sigma Scan" program.

Nucleolar Silver Staining of Whole-mount Yeast Cells

This procedure is a modification of the methods used to study the mammalian nucleolus (23, 40). Pre-fixed, whole-mount yeast cells (9) on polylysine-coated microscope slides were incubated at R.T. for 2–3 h in the presence of modified Schiffs base reagent. The cells were rinsed with dH_2O, and then 0.2 ml of 50% $AgNO_3$ was applied to the cells. These slides were incubated in a moist chamber at 55°C for 2–3 h. After this incubation, the slides were rinsed with 2 ml of dH_2O, and then 0.1 ml of ammonical silver (20) was applied to the cell and incubated at R.T. for 5 min. The solution was rinsed off with 0.2 ml of dH_2O followed by the application of 0.1 ml of the formaldehyde developer. The cells were observed in the microscope to monitor the development of the nucleolar-specific silver stain. When sufficient staining was achieved the slides were rinsed with 6–8 ml of dH_2O, and then mounted in one drop of buffered 90% glycerol (10). A coverslip was attached to the slide with clear fingernail polish. Slides were observed on a Zeiss standard microscope equipped with Nomarski DIC and epifluorescent optics. Pictures were recorded on Kodak Tri-X pan 35-mm film which was developed by the Diafine two-step development system.

Nucleolar-specific Silver Staining of an SDS–Polyacrylamide Gel

This procedure was a modification of the methods described in reference 23. After the protein samples were resolved by electrophoresis on an SDS–12% polyacrylamide gel (22), the gel was rinsed in dH_2O three times and placed in 0.9 N acetic acid for 12–16 h. After three more washes with dH_2O, the gel was then soaked for 1–2 h at R.T. in 0.1 M Na_2SO_4/0.005 M $Na_2B_4O_7$. Three dH_2O washes of the gel were then followed by a 12–16-h incubation at 50°C in 12.5% aqueous silver nitrate. After this incubation the gel was rinsed in dH_2O three times and the stain was developed at 50°C in 3% formaldehyde developer. The development was closely monitored to prevent overstaining. The development was stopped by rinsing with dH_2O and storing in 7% acetic acid. The stained gel was immediately photographed.

SDS–Polyacrylamide Gels and Protein Visualization

SDS-PAGE was done by the method described by Laemmli (22) with a 12% resolving gel. To visualize the entire content of a protein sample loaded on the SDS–polyacrylamide gel, the gels were stained by the nonspecific silver staining method of Wray et al. (46). Antigenic proteins on the gel were identified by electroblotting the proteins to nitrocellulose as described in reference 44, and then staining by incubation with the primary antibody dilutions overnight at R.T. The primary antibody binding was revealed by the procedures provided in the ABC IgG peroxidase staining kit (Vector Laboratories, Inc.).

Immunoprecipitation

Goat anti–SSB-1 antibody was used in a modified snRNA immunoprecipitation procedure (3, 8, 25). Antiserum or preimmune serum was coupled to protein G–Sepharose by incubating 2 μl of serum with 50 μl of protein G–Sepharose 4 FF (Pharmacia Fine Chemicals) at R.T. for 1 h in 0.5–1 ml of NET-2 buffer (50 mM Tris-HCl, pH 7.4, 0.05% NP-40, 150 mM NaCl). The resin was then washed three times with 1 ml of the same buffer. 5–10 μl of 0.4% NP-40–treated yeast spheroplast, at 10 μg/μl of total protein, was added to the antibody-bound protein G–Sepharose in 0.5 ml of NET buffers of the indicated NaCl molarities (50–900 mM), and incubated for 2 h at 4°C with mixing. The resin was washed extensively with NET buffer and then phenol extracted, and the isolated RNAs were analyzed as described below.

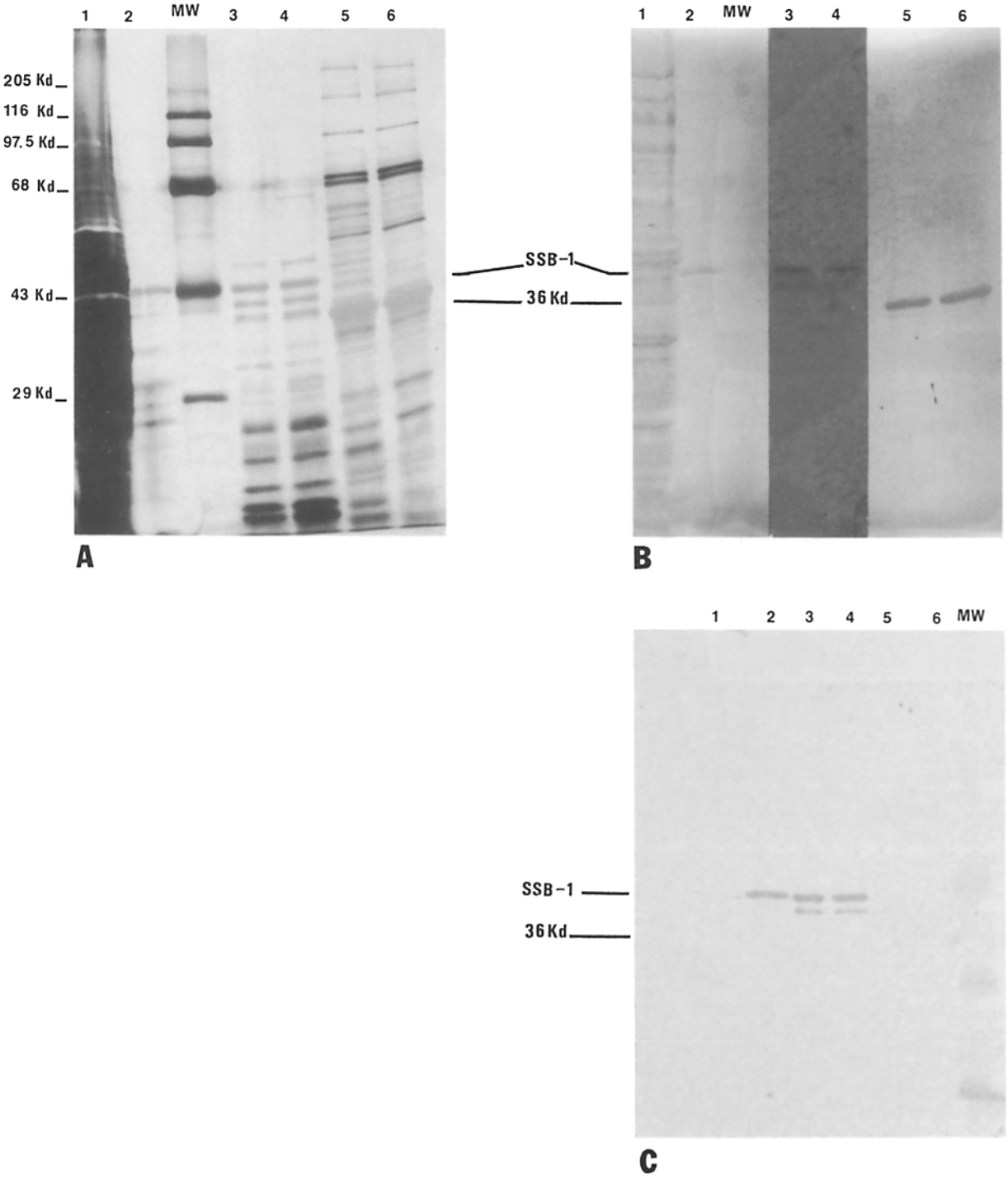

Figure 2. SDS-PAGE of selected fractions from an ssDNA cellulose column 0.2–0.8 M NaCl linear gradient revealed with nucleolar-specific stains. (Lane *1*) 100 μg of yeast cell extract. (Lane *2*) 2 μg of 80% purified SSB-1 sample used by A. Jong to clone the SSB-1 gene (18). (Lane *3*) 25 μg of pooled fractions 71–75. (Lane *4*) 25 μg of pooled fractions 65–70. (Lane *5*) 25 μg of pooled fractions 21–25. (Lane *6*) 25 μg of pooled fractions 15–20. (Lane *MW*) Protein standards with the molecular weights indicated at the side of *A*. (*A*) SDS–polyacrylamide gel stained with the nonspecific silver stain of Wray et al. (46); (*B*) SDS–polyacrylamide gel stained with the nucleolar-specific silver stain. Lanes *3* and *4* have been photographically overexposed to demonstrate that only the 43- and 38-kD bands are stained. (*C*) Anti–SSB-1 staining of an immunoblot of an equivalent gel. The primary, affinity-purified SSB-1 antibody was used at a 1:500 dilution. SSB-1 migrates with a relative molecular weight of ~43,000. The major proteolytic fragment of SSB-1 has a relative molecular weight of 38,000. SSB-36-kD migrates with a relative molecular weight of 36,000.

3'-End-Labeled RNAs

In vitro labeling of RNAs with [5'-^{32}P] pCp and T4 RNA ligase was done as described in reference 3. Immunoprecipitated RNAs were purified by phenol-chloroform extraction and ethanol precipitation in the presence of glycogen carrier (20 μg per reaction). These RNAs were added to a 10-μl reaction containing cytidine 3',5'-[5'-^{32}P]bisphosphate (40 μCi; 3,000 μCi/mmol) in 30 mM Hepes-KOH, pH 8.3, 6 mM $MgCl_2$, 4 mM DTT, 15 μM Na-ATP, 10% DMSO, and 3% PEG-8000. The ligation reaction was carried out for 4 h at R.T. in the presence of 4 U of T4 RNA ligase and 32 U of RNasin. After ethanol precipitation, the RNAs were separated by electrophoresis on a 7.5% polyacrylamide–8 M urea gel in a buffer containing Tris-borate and Na_2-EDTA (TBE) with an acrylamide/bisacrylamide ratio of 29:1, and subjected to autoradiography.

Northern Blot Analysis

Alternatively, unlabeled RNAs from the gel were electroblotted onto GeneScreen membrane (New England Nuclear) in 0.25× TBE buffer for 40 min at 4°C, and UV irradiated for 10 min to fix the RNA to the membrane (3). Prehybridization and hybridization of blots were carried out at 42°C in a solution containing 50% formamide, 25 mM sodium phosphate, pH 6.5, 6× SSC, 5× Denhardt's solution, 0.5% SDS, and 0.1 mg/ml calf thymus DNA. After hybridization, membranes were washed two times for 5 min each in 2× SSC and 0.5% SDS; two times for 5 min each in 2× SSC and 0.1% SDS at R.T.; and finally followed by 30 min at 60°C in 2× SSC. DNA fragments containing U1, U2, U5, snR10, and snR17 genes were used for the synthesis of the probes by the random-primer extension method.

Results

SSB-1 Is a Nucleolar-specific, Silver-binding Protein

It had been shown by Jong et al. (19, 20) that SSB-1, a major yeast protein that binds to an ssDNA cellulose column, was an RNA-binding protein with a predominant nuclear location, most likely in the nucleolus. The two immunofluorescent staining patterns seen for SSB-1, the crescent and the halo (Fig. 1 *A*), by comparison with yeast cells stained by the nucleolar-specific silver stain procedure (Fig. 1 *B*), were shown to be two views of a single nuclear feature (20), this feature being a caplike structure occupying approximately one third of the nuclear volume. The two staining patterns for SSB-1 are consistent with the appearance of the "dense crescent" of yeast (37), which has been demonstrated to be the yeast nucleolus. In an attempt to verify that this pattern was a result of SSB-1 staining, a strain of yeast (K4) that overproduced SSB-1 protein approximately threefold over wild-type levels (18) was examined by optical microscopy. Fig. 1, *C* and *D*, shows the results when prefixed (10), whole-mount cells of the K4 strain are stained with affinity-purified anti–SSB-1 (Fig. 1 *C*) and the nucleolar-specific silver stain procedure (Fig. 1 *D*). In both cases, staining is seen not only in the nucleolus, but also in the nucleoplasm and the cytoplasm. The coincident staining of the cytoplasm by both the SSB-1 antibody and the nucleolar-specific silver indicated that SSB-1 might be one of the yeast proteins responsible for the specific silver binding of the nucleolus.

The nucleolar-specific silver-binding capabilities of SSB-1 was thus tested directly by SDS-PAGE (Fig. 2). Fig. 2 *A*, lane *2*, shows the 32,800–mol wt SSB-1 protein migrating anomalously slow, as it normally does (18), at an M_r of 43,000-D. This protein sample of SSB-1 was obtained from A. Jong. He had used this sample to affinity purify antibodies to this 43,000-D protein which was bound to nitrocellulose after SDS-PAGE. He then used these affinity-purified SSB-1 antibodies to clone the SSB-1 gene (18). Fig. 2 *B*, lane *2*, shows the SSB-1 clearly being stained under the nucleolar-specific silver-binding conditions. Comparison of lanes *1* in Fig. 2, *A* and *B*, reveals that there are <30 proteins in a total cell extract that bind silver under the nucleolar-specific conditions. Thus SSB-1 is one of the yeast proteins responsible for the nucleolar-specific silver-binding property seen in the yeast cell.

To examine if any other ssDNA-binding proteins of yeast had the nucleolar-specific characteristic, the other fractions of a 0.2–0.8-M NaCl gradient from the ssDNA cellulose column were screened for the nucleolar-specific silver-binding property (data not shown). Three protein bands were found to bind silver under the nucleolar-specific conditions. At 0.55–0.6 M NaCl a doublet was revealed around M_r 43,000 (representative pooled fractions are in Fig. 2, lanes *3* and *4*). A single protein with an M_r of 36,000 was also observed at 0.35–0.4 M NaCl (representative pooled fractions are in Fig. 2, lanes *5* and *6*). Fig. 2 *C* is the protein from a duplicate gel to those in *A* and *B* that had been electroblotted to nitrocellulose and then immunostained with the affinity-purified anti–SSB-1 antibody. The doublet in lanes *3* and *4* was thus shown to share epitopes with SSB-1. The 43,000-D band is the intact SSB-1 protein, while the 38,000-D band is, most likely, a proteolytic fragment of SSB-1 (21). The single 36,000-D nucleolar-specific silver-binding protein does not react with the anti-SSB-1 antibody and is a different yeast nucleolar protein.

SSB-1 and Yeast Fibrillarin Colocalize

To definitely demonstrate that SSB-1 is a nucleolar protein, the protein was immunolocalized relative to a protein known to reside in one of the regions of the yeast nucleolus. This experiment thus required antibody markers to a specific nucleolar region. A rabbit polyclonal antiserum that was directed against the RNA Pol I 190-kD subunit of yeast (36) was obtained from A. Sentenac. This antibody reacted well with yeast spheroplasts in immunofluorescence microscopy (Fig. 3 *A*) and it produced the two staining patterns, the crescent and the halo, that are indicative of the yeast nucleolus (20, 37).

Unfortunately, because both the SSB-1 antibody and the anti–RNA Pol I antibody were raised in rabbits, these two antibodies could not be used together in double-label immunofluorescence experiments. A human autoimmune serum (24) against the nucleolar protein fibrillarin was thus examined for its reactivity against yeast. This antifibrillarin serum did react with a protein in a total yeast cell extract with an approximate M_r of 38,000. Schimmang et al. have recently cloned and sequenced the yeast gene for fibrillarin (35). This 38,000-M_r band for the human autoimmune serum was coincident on immunoblots with 38,000-M_r band revealed by a mouse mAb raised against mammalian fibrillarin (data not shown). The antifibrillarin mAb (31), a gift from E. Tan, did not work in immunofluorescence microscopy for yeast. Neither of these fibrillarin antisera reacted with SSB-1 or the SSB-36-kD nucleolar-specific, silver-binding protein described above (data not shown). The human autoimmune serum to fibrillarin worked well on yeast spheroplasts at an immunofluorescent level (7) and by double-label immunofluorescence microscopy with the RNA Pol I 190-kD antibody (Fig. 3 *B*) colocalized to the site of transcrip-

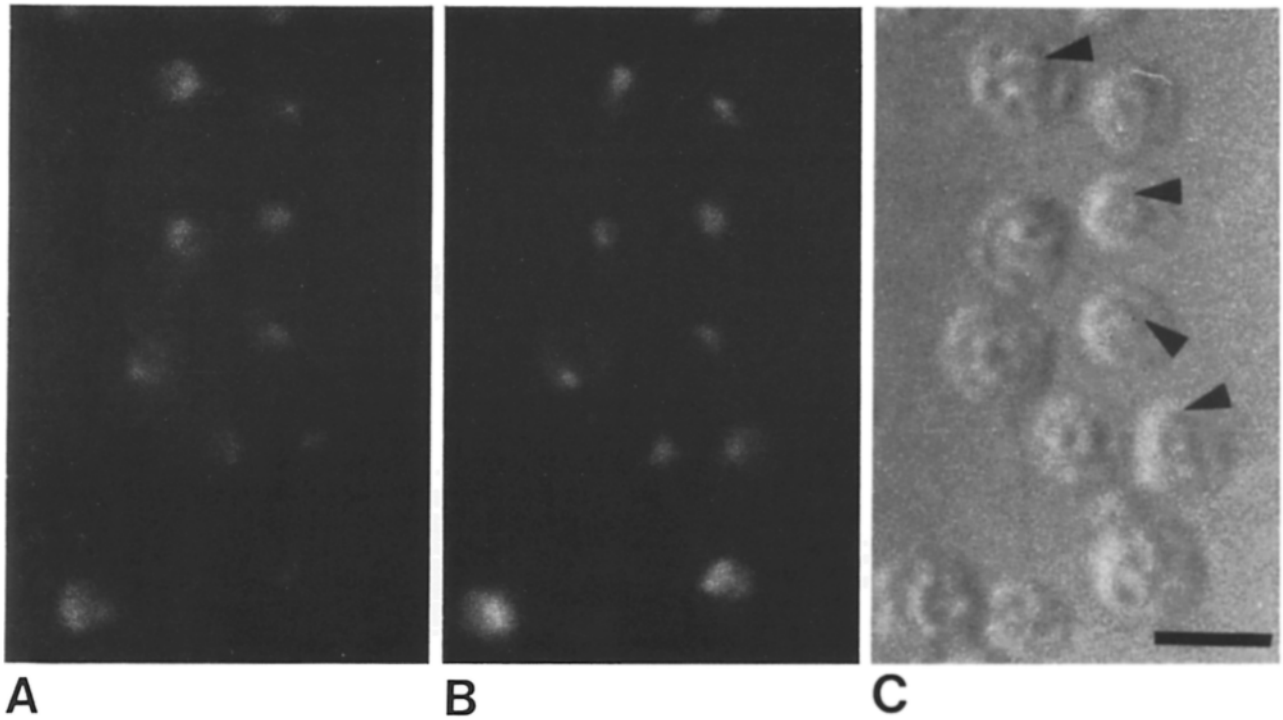

Figure 3. Double-label immunofluorescence mapping of yeast fibrillarin relative to the yeast RNA Pol I 190-kD subunit. (*A*) Postfixed, whole-mount yeast cells (10) stained with a 1:500 dilution of rabbit polyclonal serum directed against the RNA Pol I 190-kD subunit as primary antibody. The anti-RNA Pol I 190-kD location was revealed by a goat anti-rabbit IgG-TRITC conjugate viewed under the TRITC excitation wavelength. (*B*) The same cells as in *A* that have also been exposed to a fivefold concentrated serum of the ANA-nucleolar staining human autoimmune serum against fibrillarin as the primary antibody. The antifibrillarin location was revealed by a goat anti-human IgG-FITC conjugate viewed under the FITC excitation wavelength. (*C*) The same cells as in *A* and *B* viewed by Nomarski DIC to demonstrate the nucleus (*arrowheads*). Bar, 5 μm.

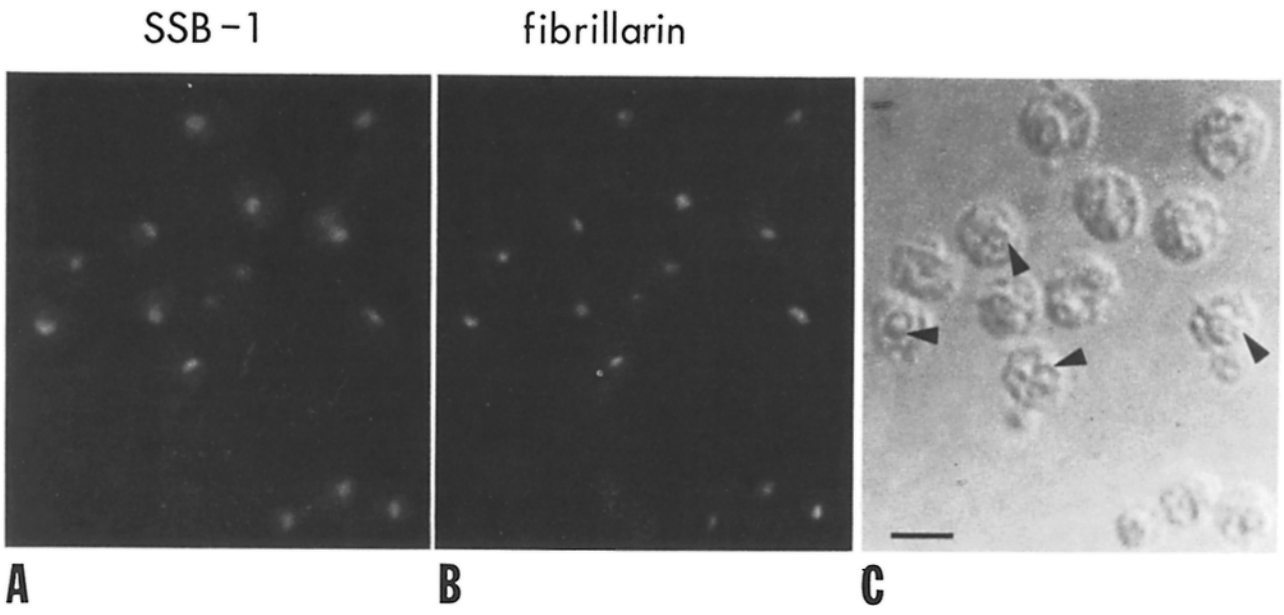

Figure 4. Double-label immunofluorescence mapping of SSB-1 relative to fibrillarin. (*A*) Postfixed, whole-mount yeast cells incubated with affinity-purified rabbit IgG against SSB-1. The anti-SSB-1 location was revealed with goat anti-rabbit-FITC conjugate viewed under FITC excitation wavelengths. (*B*) The same cells as in *A* also exposed to fivefold concentration of the antifibrillarin human autoimmune serum (Sigma Chemical Co.) The antifibrillarin location was revealed with goat anti-human IgG-TRITC conjugate viewed under TRITC excitation wavelength. (*C*) The same cells as in *A* and *B* viewed by Nomarski DIC to demonstrate the location of the nucleus (*arrowheads*). Bar, 5 μm.

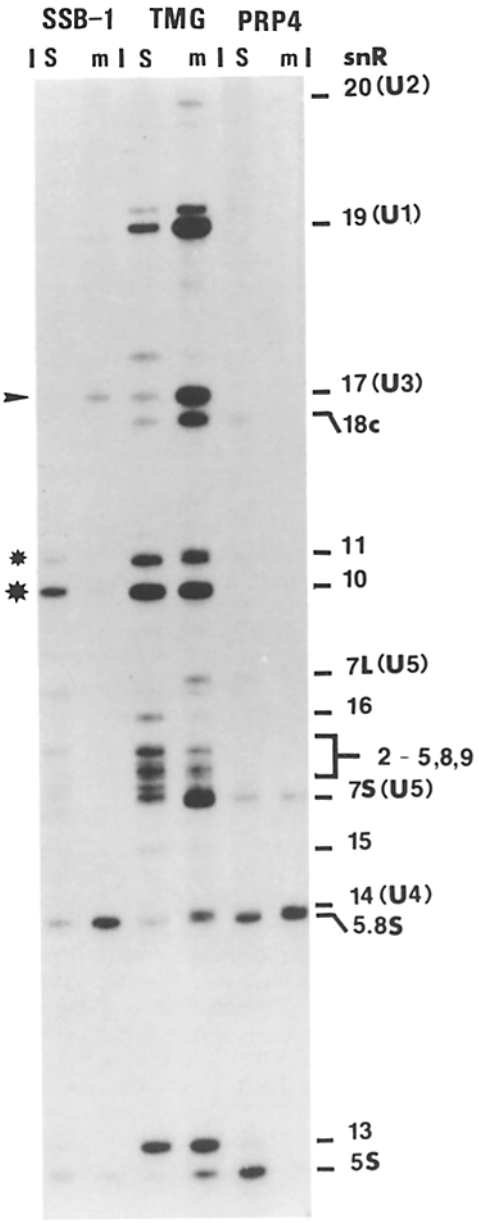

Figure 5. 3′ end-labeled snRNAs immunoprecipitated from yeast extracts. Lanes marked *SSB-1* were immunoprecipitated with an antibody raised in a goat to the yeast SSB-1 protein. This antibody gives the same staining pattern of the yeast nucleolus as the rabbit anti–SSB-1 antibody. Although the goat SSB-1 antibody fluorescent staining is weaker than the rabbit SSB-1 antibody. The goat antibody works better for this precipitation experiment. The lanes marked *TMG* were immunoprecipitated with an antibody directed against the trimethyl guanosine "cap." The lanes marked *PRP4* were immunoprecipitated with a rabbit antibody directed against the PRP4 protein that is intimately involved in the pre-mRNA splicing process and precipitates the yeast snRNAs U4 (snR14) and the two forms of U5 (snR7L and 7S). All of these immunoprecipitations were done at 150 mM NaCl. The lanes labeled *S* are precipitations done with spheroplasted yeast extract. The lanes labeled *m* are precipitation experiments done using the pre-mRNA splicing extract 40P (8). The yeast snRNAs are labeled with both the numbers given them by Riedel et al. (32), and with the mammalian *U* equivalents when appropriate. The arrowhead indicates the position of snR17 (U3). The small and large stars indicate the positions of snR11 and snR10, respectively.

tion for rRNA; i.e., the fibrillar region of the yeast nucleolus. The human autoimmune serum to fibrillarin was thus used in double-label immunofluorescence microscopy with the rabbit anti–SSB-1 serum to map SSB-1 relative to the fibrillar region of the yeast nucleolus. Fig. 4 shows the results of the double-label immunofluorescent mapping of SSB-1 (Fig. 4 *A*) compared with fibrillarin (Fig. 4 *B*). The two proteins colocalized in the yeast nucleolus. By overlaying micrographs, the relative positions of the staining patterns for the respective proteins were determined to be in the same locations in the yeast nucleus, i.e., the nucleolus. When the digitized areas of antifibrillarin staining and anti–SSB-1 staining from 147 cells were compared, the SSB-1 occupied 48% of the nuclear area while the fibrillarin only occupied 32% of the nuclear area. The area of yeast nucleolus stained by the RNA Pol I 190-kD antibody also only covered 33% of the nuclear area.

SSB-1 Antibodies Will Immunoprecipitate snRNAS

It has been shown that antisera to the nucleolar protein fibrillarin will immunoprecipitate not only the U3 snRNA but also other snRNAs in both yeast (42) and mammals (45). The U3 snRNA (snR17 in yeast) is an essential gene and it has been implicated in the early events in either ribosome assembly or pre-rRNA maturation. Fibrillarin's association with U3 snRNA, forming a nucleolar snRNP particle, suggests that fibrillarin is also involved in these nucleolar processes. Since SSB-1 binds to single-strand polynucleotides (DNA and RNA), yeast cell extracts were immunoprecipitated with an SSB-1 antibody to determine if there were any snRNA associations. The anti–SSB-1–precipitated RNAs were examined by 3′ end-labeling them with 32pCp by T4 RNA ligase and then separating the products by electrophoresis on a denaturing polyacrylamide gel (Fig. 5). Fig. 5, (*SSB-1*, lane *S*), shows the RNAs immunoprecipitated by the SSB-1 antibody.

Based on their comigration with the capped snRNAs precipitated with ant–trimethyl guanosine "cap" (Fig. 5, *TMG*) reported for yeast (32, 33) and on Northern blot analysis, the two major snRNAs that coprecipitated with SSB-1 are snR10 and snR11, with snR10 being predominant. Some smaller RNAs of 170–190 nucleotides were also moderately precipitated (Fig. 5, bracket 2–5, 8, 9). 5.8S and 5S rRNA, because of their abundance in the cell, appear in varying amounts in all immunoprecipitations, even those with preimmune serum (see Fig. 6 *A*, *Preimmune*) and are thus not considered to be specific interactions. This snRNA precipitation pattern was reproducible as long as yeast spheroplasts were used as the cell extract in the manner described in Materials and Methods. When pre-mRNA splicing extract 40P (8) was used for SSB-1 immunoprecipitation (for this extract was known to contain the yeast "capped" snRNAs), the SSB-1/snRNA associations changed (Fig. 5, *SSB-1*, lane *m*). snR17, which only weakly precipitated with the SSB-1 antibody in spheroplast extracts (Fig. 5, *SSB-1*, lane *S*), showed a stronger association with SSB-1 in pre-mRNA splicing extracts (Fig. 5, *SSB-1*, lane *m*) than snR10 and snR11. The 170–190 nucleotide-sized RNAs were eliminated. Comparison of spheroplast extract and pre-mRNA splicing extracts, (*TMG*, lanes *S* and *m*), which had been immunoprecipitated with anti-TMG, showed that there were approximately the

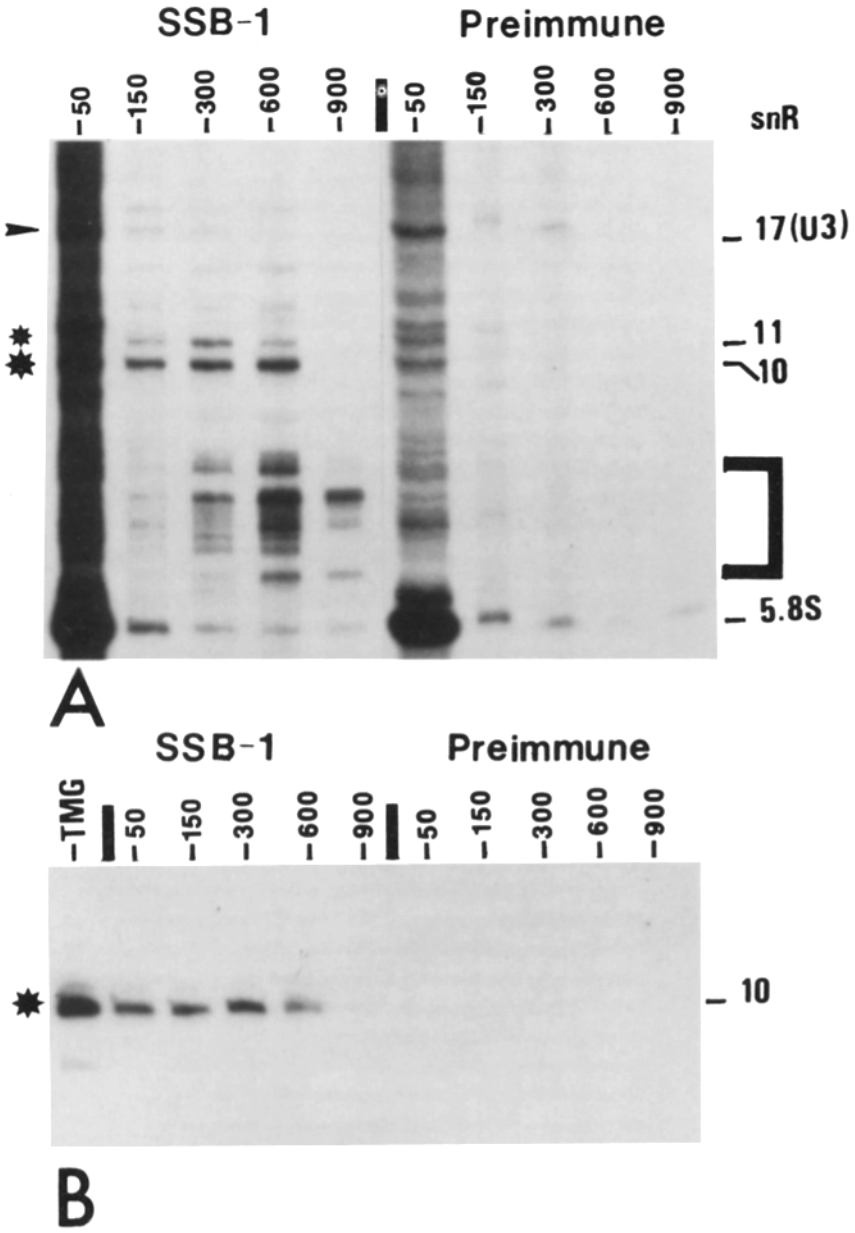

Figure 6. NaCl concentration curve for the anti–SSB-1 immunoprecipitation. Lanes marked *SSB-1* were precipitated with a goat antibody raised against SSB-1. Lanes marked *Preimmune* were precipitated with serum from the goat before the injection of the SSB-1 protein as a control for the immune specificity. Lane headings (*50–900*) refer to the concentration of NaCl (mM) used for the precipitation experiment. Lane *TMG* is an immunoprecipitation done with the anti-cap antibody at 150 mM NaCl. (*A*) 3′ end-labeled snRNAs separated on a urea-containing polyacrylamide gel. The arrowhead indicates the position at which snR17 (U3) migrates on the gel. The small and large stars indicate the positions of snR11 and snR10, respectively. The bracket denotes the region of the gel at which RNAs of the 175–190-nucleotide size range migrate. (*B*) Northern blot analysis of the snRNAs from the same experiment as in *A*. Shown is the result of the blot hybridized with an snR10 probe. The same blot probed for snR17 (U3) showed some snR17 in 50–300 mM NaCl (data not shown). The presence of snR17 in the 150–300 mM NaCl lanes can be seen in *A* (*arrowhead*). This same blot probed for U1, U2, and U5 showed binding for these snRNAs only in lane *TMG* (data not shown).

same quantities of precipitable capped snRNAs in each precipitate. The differences in the immunoprecipitability of the snRNAs in the two experiments with the SSB-1 antibody appears to be due to differences in the preparation of the extracts. The pre-mRNA splicing extract used here, referred to as 40P, was prepared by 40% ammonium sulfate precipitation of cell lysates. 40% ammonium sulfate is equivalent to 2.4 M salt. Whereas the yeast spheroplasts were never exposed to greater than physiological salt concentrations remaining intact with 1.2 M sorbitol and 100 mM salt until use.

This salt-dependent variability of snRNAs binding to SSB-1 can be clearly seen when the immunoprecipitation was done as a function of increasing NaCl concentrations (Fig. 6 *A*, *SSB-1*, lanes *50–900*). snR10 and snR11 (Fig. 6, *large star* and *small star*, respectively) immunoprecipitability begins to decrease after 0.6 M NaCl and was completely lost at 0.9 M. The immunoprecipitability of the RNAs between 170 and 190 nucleotides in length, however, increased with increasing salt. These snRNA associations were specific for SSB-1 because immunoprecipitations under the same conditions with preimmune serum showed no snRNAs above 50 mM NaCl (Fig. 6 *A*, *Preimmune*, lanes *50–900*). Fig. 6 *B* is the Northern blot analysis of the same NaCl concentration curve experiment seen in Fig. 6 *A*. It was hybridized with a DNA probe for snR10 and it shows the same decrease of snR10 binding to SSB-1 with increasing salt. When this blot was rehybridized with a probe for snR17, the yeast U3 equivalent, only a small amount of snR17 was seen associated with SSB-1 at the lower salt concentration (data not shown). The correctly sized band for snR17 can be seen in the 3′ end-labeled experiment showing the same snR17 association (Fig. 6 *A*, *SSB-1*, lanes *150* and *300*, *arrowhead*). The reassociation of SSB-1 to snR17 seen for 40P must require the high ammonium sulfate concentration and longer exposure times used in the preparation of the pre-mRNA splicing extracts.

Discussion

It has been demonstrated here, by nucleolar-specific silver staining and double-label immunofluorescence microscopy with antibodies to RNA Pol I, fibrillarin, and SSB-1, that SSB-1 protein is one of the yeast nucleolar-specific, silver-binding proteins and is located in the nucleolus. The immunoprecipitation experiments show that at 150 mM NaCl, physiological salt concentration, SSB-1 is strongly associated with snR10. Tollervay (42) reported that snR10 is involved in pre-rRNA processing. By association with snR10, SSB-1 is also implicated in that process. The immunolocalization of SSB-1 in the nucleolus supports this conclusion. Such a conclusion for SSB-1 function and snR10 association is further corroborated by the finding that a frameshift mutant of SSB-1 is not lethal in the yeast cell (18) and is similar to the viable phenotype resulting from the snR10 gene disruption (43). A total deletion of the SSB-1 gene from the yeast cell is also still viable although the growth rate is slowed and the unprocessed 35S rRNA accumulates when glycerol is used as a carbon source (Clark M. W., unpublished data). This change in growth rate and 35S rRNA accumulation is similar to the results obtained with the snR10 gene disruption (43).

The salt-dependent variability in snRNA association to SSB-1 is a phenomenon that has been demonstrated in mammalian cells. Using the antibody to the nucleolar protein, fibrillarin, a decrease in immunoprecipitability of U3 snRNA is seen as the NaCl concentration increases. At higher salt concentrations antifibrillarin-dependent immunoprecipitability is greater for the snRNAs U8 and U13 (45). These variable snRNA associations to these nucleolar proteins with varying salt concentration could (*a*) represent a disruption of the primary interaction between the protein and its snRNA that subsequently allows protein association with secondary snRNA targets or (*b*) reflect changes in affinities and the nucleolar snRNP composition resulting from different molecular states of the rRNA maturation process. The RNAs of the 170–190-nucleotide range that increase their SSB-1 association with higher salt fit the size range for the snRNAs (snR3, snR4, snR8, and snR13) that Tollervey (42) has shown to hybridize to the 35S rRNA. Verification of the identities of these snRNAs is under way. If the identities of these 170–190-nucleotide-long snRNAs are confirmed, the involvement of these salt-dependent association with SSB-1 in rRNA processing will need to be pursued.

The nucleolus in yeast, unlike that of the higher organisms, is a single nuclear structure that appears as a dense crescent, filling about one third of the nuclear volume. The yeast nucleolus is juxtaposed to the inner membrane of the nuclear envelope (5, 13, 20, 29). Early electron micrographic studies have shown that the yeast nucleolus is made up of only a fibrillar region and a granular component. No region corresponding to the dense fibrillar component is seen in yeast (5, 13). Benavente et al. (4) have reported this region to be involved in the reassembly of the mammalian nucleolus during late telophase. The lack of a dense fibrillar region in yeast may come from the fact that the yeast nucleolus never completely disassembles during cell cycle, so it does not require a mechanism for reassembly. The yeast nucleolus thus requires some strategy to provide entrance of new nucleolar and ribosomal components into the continually functioning nucleolus. Since rRNA processing has been shown to occur in the granular region of the nucleolus (39), it is likely that SSB-1 will be found to reside in the equivalent region of the yeast nucleolus. This proposed localization of SSB-1 is strengthened by a recent report by Potashkin et al. (29) in their investigations of the nuclei of the fission yeast *Schizosaccharomyces pombe*, and the budding yeast *S. cerevisiae*. They first show that, when using the nucleolar-specific silver stain at an EM level, the granular region of the yeast nucleolus stains specifically with silver. These nucleolar-specific silver-staining patterns reach to the inner membrane of the nuclear envelope. Second, they show by immunoelectron microscopy with an antibody directed against the 2,2,7-trimethyl-guanosine cap structure found at the 5′ termini of the snRNAs that this antibody predominantly stains the granular region of the yeast nucleolus. This, of course, is the expected result because the majority of the snRNAs in yeast appear to be involved in rRNA processing (42; Clark, M. W., personal observation). Thus by the silver staining and snRNA location in the granular region of the yeast nucleolus, it follows that SSB-1 should also reside there. The reduced area of fluorescent antibody staining for fibrillarin and the RNA Pol I 190-kD protein (33%) relative to SSB-1 (48%) implies that the fibrillar region of the yeast nucleolus is more compact than the granular region. The EM work of Potashkin et al. (29) also supports this immunofluorescence finding. They report that the fibrillar centers of the yeast nucleolus, the sites of rRNA transcription, are relatively small in size and are positioned in the more internal regions of the nucleolus and the nucleus. While the granular region of the yeast nucleolus, the area of pre-rRNA processing and pre-ribosome assembly, is located in the periphery of the nucleus, juxtaposed to the nuclear envelope. This organization of the nucleolus leaves the rDNA accessible to the nucleoplasm and the components of the RNA Pol I and RNA Pol III complexes necessary for the transcription of the rRNAs.

The specific compartmentalization of nucleolar processes in the nucleus, as well as the nucleolus, dictates the conclusion that there is some sort of cellular machinery that generates this nuclear and nucleolar organization. The localization of the nucleolar proteins in specific regions of the nucleolus indicates that there must be signals in these proteins that determine their final nucleolar destination. The first amino acid sequence that targets a protein to the nucleolus, i.e., a nucleolar targeting signal, has been found in the $p27^{x-iii}$, HTLV-1 protein (38). SSB-1 has no amino acid regions directly homologous to this mammalian nucleolar targeting signal. The association of SSB-1 with snR10 to make a nucleolar snRNP particle may make the nucleolar targeting signal for SSB-1 more complicated than a simple amino acid signal. snRNPs of the pre-mRNA splicing process have been demonstrated to first assemble in the cytoplasm and then be transported into the nucleus (12). The same situation might occur for the yeast nucleolar snRNPs. Steitz et al. (41) have shown that mammalian ribosomal protein L5 must be associated with the 5S rRNA before either of these macromolecules can enter the nucleolus. The mislocalization of SSB-1 in the cytoplasm as a result of overproduction (Fig. 1, *C* and *D*) can be explained if SSB-1 must first be bound with snR10 or snR11 before proper nucleolar targeting can take place. Fortunately, the deletion of the gene for snR10

or SSB-1 from yeast is not a lethal event (43; Clark, M. W., personal observation); thus any snR10 requirement for SSB-1 nucleolar targeting signal can be directly tested in situ.

The nucleolar-specific staining procedures have identified some of the other yeast nucleolar proteins. The SSB-36-kD protein is a major yeast nucleolar protein and it will be further studied. Since SSB-36-kD shares many of the same nucleolar-specific characteristics as SSB-1, it is likely that SSB-36-kD is also an integral part of a nucleolar snRNP that is involved in one of the steps of rRNA maturation or ribosome assembly. When antibodies to SSB-36-kD become available this snRNA association will be tested.

We thank A. Sentenac at Service de Biochimie, Gif-sur-Yvette, Cedex, France and E. Tan at Scripps Clinic, La Jolla, Ca, for the antisera to RNA Pol I 190-kD protein and fibrillarin (mouse monoclonal), respectively. We thank A. Jong at the University of Southern California, Los Angeles, CA, and M. Cusick at Texas A & M University, College Station, TX, for the purified SSB-1 protein and ssDNA cellulose column fractions. We thank E. Shuster at the University of California, San Francisco, CA for the clones of the yeast snRNAs and E. Jones at Carnegie-Mellon University, Pittsburgh, PA, for yeast strain EJ101.

M. W. Clark was supported by the National Institutes of Health (NIH) postdoctoral fellowship 5732 GM 1008-02. M. L. R. Yip was supported by Howard Hughes Institute Doctoral Fellowship in Biological Sciences. J. Campbell was supported by NIH grant GM 25588. J. Abelson was supported by NIH grant GM 3267.

Received for publication 27 March 1990 and in revised form 27 July 1990.

References

1. Aris, J. P., and G. Blobel. 1988. Identification and characterization of a yeast nucleolar protein that is similar to a rat liver nucleolar protein. *J. Cell Biol.* 107:17–31.
2. Bandziulis, R. J., M. S. Swanson, and G. Dreyfuss. 1989. RNA-binding proteins as developmental regulators. *Genes & Dev.* 3:431–437.
3. Banroques, J., and J. Abelson. 1989. PRP4: a protein of the yeast U4/U6 small nuclear ribonucleoprotein particle. *Mol. Cell. Biol.* 9:3710–3719.
4. Benavente, R., K. M. Rose, G. Reimer, B. Hugle-Dorr, and U. Scheer. 1987. Inhibition of nucleolar reformation after microinjection of antibodies to RNA polymerase I into mitotic cells. *J. Cell Biol.* 105:1483–1491.
5. Byers, B. 1981. Cytology of the yeast life cycle. *In* Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance. J. N. Strathern, E. W. Jones, and J. R. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 59–96.
6. Chan, E. K. L., K. F. Sullivan, and E. M. Tan. 1988. Ribonucleoprotein SS-B/La belongs to a protein family with consensus sequence for RNA-binding. *Nucleic Acids Res.* 17:2233–2244.
7. Chang, T.-H., M. W. Clark, A. J. Lustig, M. E. Cusick, and J. Abelson. 1988. RNA11 protein is associated with the yeast spliceosome and is localized in the periphery of the cell nucleus. *Mol. Cell. Biol.* 8:2379–2393.
8. Cheng, S.-C., and J. Abelson. 1987. Spliceosome assembly in yeast. *Genes & Dev.* 1:1014–1027.
9. Chooi, W. Y., and K. R. Leiby. 1981. An electron microscopic method for localization of ribosomal proteins during transcription of ribosomal DNA: a method for studying protein assembly. *Proc. Natl. Acad. Sci. USA.* 78:4823–4827.
10. Clark, M. W., and J. Abelson. 1987. The subnuclear localization of tRNA ligase in yeast. *J. Cell Biol.* 105:1515–1526.
11. Fakan, S., and E. Puvion. 1980. The ultrastructural visualization of nucleolar and extranucleolar RNA synthesis and distribution. *Int. Rev. Cyto.* 65:255–299.
12. Feeney, R. J., R. A. Sauterer, J. L. Feeney, and G. W. Zieve. 1989. Cytoplasmic assembly and nuclear accumulation of mature small nuclear ribonucleoprotein particles. *J. Biol. Chem* 264:5776–5783.
13. Hadjiolov, A. A. 1984. The Nucleolus and Ribosome Biogenesis. Springer-Verlag, New York Inc., New York. 1–30.
14. Ho, Y.-S., C.-Z. Huang, and Y.-C. Tu. 1987. A monoclonal antibody (HO-No-I) with specificity for a human nucleolar protein. *Br. J. Exp. Pathol.* 68:839–845.
15. Hugle, B., R. Hazan, U. Scheer, and W. W. Franke. 1985. Localization of ribosomal protein S1 in the granular component of the interphase nucleolus and its distribution during mitosis. *J. Cell Biol.* 100:873–886.
16. Hugle, B., U. Scheer, and W. W. Franke. 1985. Ribocharin: a nuclear Mr 40,000 protein specific to precursor particles of the large ribosomal subunit. *Cell.* 41:615–627.
17. Hurt, E. C., A. McDowall, and T. Schimmang. 1988. Nucleolar and nuclear envelope proteins of the yeast Saccharomyces cerevisiae. *Eur. J. Cell Biol.* 46:554–563.
18. Jong, A. Y. S., and J. L. Campbell. 1986. Isolation of the gene encoding yeast single-stranded nucleic acid binding protein 1. *Proc. Natl. Acad. Sci. USA.* 83:877–881.
19. Jong, A. Y. S., R. Aebersold, and J. L. Campbell. 1985. Multiple species of single-stranded nucleic acid-binding proteins in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 260:16367–16374.
20. Jong, A. Y. S., M. W. Clark, M. Gilbert, A. Oheim, and J. L. Campbell. 1987. *Saccharomyces cerevisiae* SSB1 protein and its relationship to nucleolar RNA-binding proteins. *Mol. Cell. Biol.* 7:2947–2955.
21. LaBonne, S. G., and L. B. Dumas. 1983. Isolation of a yeast single-strand deoxyribonucleic acid binding protein that specifically stimulates yeast DNA polymerase I. *Biochemistry.* 22:3214–3219.
22. Laemmli, U. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
23. Lischwe, M. A., K. Smetana, M. O. J. Olson, and H. Busch. 1979. Proteins C23 and B23 are the major nucleolar silver binding proteins. *Life Sci.* 25:701–708.
24. Lischwe, M. A., R. L. Ochs, R. Reddy, R. G. Cook, L. C. Yeoman, E. M. Tan, M. Reichlin, and H. Busch. 1985. Purification and partial characterization of a nucleolar scleroderma antigen (Mr = 34,000; pI, 8.5) rich in N^g, N^g-dimenthylarginine. *J. Biol. Chem.* 260:14304–14310.
25. Lossky, M., G. J. Anderson, S. P. Jackson, and J. Beggs. 1987. Identification of a yeast snRNP protein and detection of snRNP-snRNP interactions. *Cell.* 51:1019–1026.
26. Ochs, R. L., M. A. Lischwe, W. H. Spohn, and H. Busch. 1985. Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. *Biol. Cell.* 54:123–134.
27. Parker, K. A., and J. A. Steitz. 1988. An in vitro interaction between the human U3 snRNP and 28S rRNA sequences near the alpha-sarcin site. *Nucleic Acids Res.* 22:10493–10509.
28. Parker, R., T. Simmons, E. O. Shuster, P. G. Siliciano, and C. Guthrie. 1988. Genetic analysis of small nuclear RNAs in *Saccharomyces cerevisiae*: viable sextuple mutant. *Mol. Cell. Biol.* 8:3150–3159.
29. Potashkin, J. A., R. J. Derby, and D. L. Spector. 1990. Differential distribution of factors involved in pre-MRNA processing in the yeast cell nucleus. *Mol. Cell Biol.* 10:3524–3534.
30. Query, C. C., R. C. Bently, and J. D. Keene. 1989. A common RNA recognition motif identified within a defined U1 RNA binding domain on the 70K U1 snRNP protein. *Cell.* 57:89–101.
31. Reimer, G., K. M. Pollard, C. A. Penning, R. L. Ochs, M. A. Lischwe, H. Busch, and E. M. Tan. 1987. Monoclonal autoantibody from NZB/NZW F1 mouse and some human scleroderma sera target a Mr 34,000 nucleolar protein of the U3-ribonucleoprotein particle. *Arthritis Rheum.* 30:793–800.
32. Riedel, N., J. A. Wise, H. Swerdlow, A. Mak, and C. Guthrie. 1986. Small nuclear RNAs from *Saccharomyces cerevisiae*: unexpected diversity in abundance, size, and molecular complexity. *Proc. Natl. Acad. Sci. USA.* 83:8097–8101.
33. Riedel, N., S. Wolin, and C. Guthrie. 1987. A subset of yeast snRNA's contains functional binding sites for the highly conserved Sm antigen. *Science (Wash. DC).* 235:328–331.
34. Scheer, U., and K. M. Rose. 1984. Localization of RNA polymerase I in interphase cells and mitotic chromosomes by light and electron microscopic immunocytochemistry. *Proc. Natl. Acad. Sci. USA.* 81:1431–1435.
35. Schimmang, T., D. Tollervey, H. Kern, R. Frank, and E. C. Hurt. 1989. A yeast nucleolar protein related to mammalian fibrillarin is associated with small nucleolar RNA and is essential for viability. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:4015–4024.
36. Sentenac, A. 1985. Eukaryotic RNA Polymerases. *CRC Crit. Rev. Biochem.* 18:31–90.
37. Sillevis-Smitt, W. W., J. M. Vlak, I. Molenaar, and Th. H. Rozin. 1973. Nucleolar function of the dense crescent in the yeast nucleus. *Exp. Cell Res.* 80:313–321.
38. Siomi, H., H. Shida, S. H. Nam, T. Nosaka, M. Maki, and M. Hatanaka. 1988. Sequence requirements for nucleolar localization of human T cell leukemia virus type I pX protein, which regulates viral RNA processing. *Cell.* 55:197–209.
39. Sommerville, J. 1986. Nucleolar structure and ribosome biogenesis. *Trends Biochem. Sci.* 11:438–442.
40. Spector, D. L., R. L. Ochs, and H. Busch. 1984. Silver staining, immunofluorescence, and immunoelectron microscopic localization on nucleolar phosphoproteins B23 and C23. *Chromosoma (Berl.).* 90:139–148.
41. Steitz, J. A., C. Berg, J. P. Hendrick, H. LaBranche-Chabot, A. Metspalu, J. Rinke, and T. Yanio. 1988. A 5S rRNA/L5 complex is a precursor to ribosome assembly in mammalian cells. *J. Cell Biol.* 106:545–556.
42. Tollervey, D. 1987. A yeast small nuclear RNA is required for normal processing of pre-ribosomal RNA. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:4169–4175.
43. Tollervey, D., and C. Guthrie. 1985. Deletion of a yeast small nuclear RNA

gene impairs growth. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:3873–3878.
44. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.
45. Tyc, K., and J. A. Steitz. 1989. U3, U8 and U13 compromise a new class of mammalian snRNPs localized in the cell nucleolus. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3113–3119.
46. Wray, W., T. Boulikas, V. D. Wray, and R. Hancock. 1981. Silver staining of proteins in polyacrylamide gels. *Anal. Biochem.* 118:197–203.
47. Yang, C. H., E. J. Lambie, J. Hardin, J. Craft, and M. Snyder. 1989. High order structure is present in the yeast nucleus: autoantibody probes demonstrate that the nucleolus lies opposite the spindle pole body. *Chromosoma (Berl.).* In press.